

## Phosphorus content and distribution and the activity of phosphatases in Arenosols in a forest affected by long-term exposure to the effects of the Anwil S.A. nitrogen works in Włocławek

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**Abstract.** This paper presents the results of research examining the soil content of total and available phosphorus ( $P_{E-R}$ ), taking into consideration the activity of alkaline and acid phosphatases. For this study, three soil profiles were sampled in Arenosols at a distance of 0.8, 2.0 and 2.5 km from a nitrogen fertiliser manufacturer, Anwil S.A. A control profile was taken from the Tuchola Forest. The soils' reaction ranged from acidic to very acidic. The humus content in the surface horizons of the sampled profiles was average (1.26–2.61%). The lowest  $P_{E-R}$  content was found in the profile taken closest (0.8 km – profile I) to the factory. The distribution index (DI) calculated for available phosphorus pointed towards moderate accumulation, whilst at the same time, the availability index (IM) confirmed low availability, especially in profile I. The activity of alkaline and acid phosphatases, which are the enzymes responsible for phosphorus transformation in the soil, varied depending on the distance from the nitrogen works. The inhibition of alkaline phosphomonoesterases and the stimulation of acid esterases, which were both connected to the examined soil reaction, were observed. The activity of phosphatases, as well as total and available phosphorus content, decreased steeply along the soil profiles. Furthermore, a significant correlation between organic carbon and the activity of alkaline and acid phosphatases ( $r = 0.94$ ,  $p < 0.05$  and  $r = 0.67$ ,  $p < 0.05$ , respectively), as well as between the content of  $P_{E-R}$  and the activity of alkaline phosphatase ( $r = 0.67$ ,  $p < 0.05$ ) were recorded. The results suggest the need for further research and monitoring of the Arenosols in the forest affected by the nitrogen works.

**Keywords:** forest soil, enzymes, phosphorus

### 1. Introduction

The rapid development of industry in the world is one of the main causes of adverse ecological changes occurring in the environment. These changes are usually directly proportional to the development of industry. This results in a need to assess the extent of environmental degradation, particularly of areas located near industry (Bielińska, Ligęza 2010). In the vicinity of large emitters, the forest and soil act as filtering and buffer systems for dust and gas emissions. If the storage capacity of the plants and soil is exceeded, the ecosystem becomes degraded (Kowalkowski, Kopron 2006). The physical, chemical and biological parameters of soil react relatively quickly to anthropogenic environmental changes, including industrial emissions (Koper et al. 2008; Telesiński et al. 2010).

Contamination of the soil near industry, waste storage and air pollution lead to changes in the availability of nutrients, whose ions can migrate into the soil profile (Shang et al. 2012), resulting in, amongst others, eutrophication. Biological methods are amongst those used to diagnose the state of soil pollution (Hinojosa et al. 2008; Bartkowiak, Lemanowicz 2014). Measurements of activity of enzyme, such as dehydrogenases, phosphatases, ureases, and proteases, are mainly used (Kucharski et al., 2011; Januszek et al. 2014), because they are very sensitive to natural and anthropogenic environmental changes and are indications of the soil's fertility level.

The aim of this study is to determine the effect of long-term exposure to a nitrogen fertiliser manufacturer on the physical and chemical properties of rusty forest soil based on its phosphatase activity.

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## 2. Material and methods

### Study site

Anwil S.A. Nitrogen Works, established in 1966, is one of the country's largest producers of nitrogen fertilisers, manufacturing, amongst others, ammonium nitrate and calcium ammonium nitrate. It also produces suspension polyvinyl chloride, chemical products for processing in a variety of industrial sectors and agricultural products. Its manufacturing facilities are located in Włocławek (52°41'55"N, 18°58'09"E), in the Kuyavian-Pomeranian Voivodeship of the Wielkopolska-Pomeranian region. The forests near the factory are dominated by Scots pine (*Pinus sylvestris* L.), growing in a fresh mixed coniferous forest habitat (BMśw). The undergrowth is dominated by wood sorrel (*Xalis acetosella*), *Polytrichum attenuatum*, and bracken (*Pteridium aquilinum*).

To carry out the study, soil samples were taken from the mineral levels of three soil profiles in June 2010 from sites adjacent to the Anwil S.A. nitrogen factory in its impacted area. The studied profiles of rusty forest soil (belonging to the order: rusty soils *R*, type: rusty *RW*, subtype: typical rusty *RWt*) (Commission V Genesis, Classification and Cartography of Soils, PTG. 2011) were categorised by distance from the factory: profile I (level *A*, *B<sub>v</sub>*, *C*) – approximately 0.8 km to the north-west; profile II (level *A*, *AB<sub>v</sub>*, *C<sub>1</sub>*, *C<sub>2</sub>*) – approximately 2 km west; profile III (Level *A*, *AB<sub>v</sub>*, *B<sub>v</sub>*, *C*) – approximately 2.5 km from the right bank of the Vistula River to the east. The profile of the rusty forest soil control (level *A*, *AB<sub>v</sub>*, *B<sub>v</sub>*, *C*), located beyond the reach of the emissions, was taken from a fresh mixed coniferous forest (BMśw) in Szumiąca in the Tuchola Forest.

### Chemical analysis of the soil

Selected physical and chemical properties were determined of air-dried soil samples of a disturbed structure, [prepared according to the PN-ISO 11464 (1999) standard]: total organic carbon (TOC), using the Scalar Company's TOCN FORMACTS™ analyser – the results were converted into humus, total nitrogen (TN) by the Kjeldahl method (ISO 11261, 2002), the potentiometric pH in 1M KCl (PN-ISO-10390, 1997), total phosphorus (TP) by the method of Mehta et al. (1954), available phosphorus ( $P_{E-R}$ ) by the Egner–Riehm method – DL (PN-R-04023, 1996), alkaline phosphatase activity (AIP) [E.C. 3.1.3.1] and acid phosphatase activity (AcP) [E.C. 3.1.3.2] by the Tabatabai and Bremner method (1969). On the basis of the results of studying the activity of alkaline and acid phosphatases, the enzymatic indicator of the soil pH level was calculated (*AIP/AcP*) (Dick et al. 2000). To assess the availability of phosphorus, the index of mobility (*IM*) was used, which expresses the percentage of available phosphorus in the total content. The

distribution of the analysed elements in the soil profiles are described using a distribution ratio (*DI*) (Kobierski et al. 2011), as the proportion of the amount of the element in the O, A and B horizons to the amount of the element in the parent rock (horizon C), where  $DI < 1$  is the absence of anthropogenic impact,  $1 \leq DI \leq 3$  indicates moderate accumulation,  $3 \leq DI \leq 6$  – significant accumulation and  $DI > 6$  – very high accumulation.

This paper presents the arithmetic mean of the results obtained from three repetitions. The results of determining the examined characteristics were analysed using a simple correlation ( $p < 0.05$ ), which describes the degree of relationship between the various characteristics. The correlation analysis was performed using the Statistica 10 program.

## 3. Results and discussion

Based on a granulometric analysis, it was found that these soils were made up of mechanically loose and slightly loamy sands (Polish Society of Soil Science 2009). The amount of clay fraction ( $\phi < 0.002$  mm) varied in the range 1–9% (Table 1). The predominant fraction was the sand fraction, with a diameter of from 0.05 to 2.0 mm. The exchangeable acidity was in the pH range 4.09–5.41, whilst at the control site, it was 4.25–5.11 (Table 1), indicating acidic and very acidic soil. According to Bielińska et al. (2009), acidification of the soil in the vicinity of the factory was related to the long-term emission of nitrogen. Increased emissions of nitrogen to forest soils result in the release of  $H^+$  protons, which is not compensated by being bound during the mineralisation of plant material. This contributes to the secondary effects of soil acidification. The carbon content of the organic compounds in the factory-impacted soil profiles studied ranged from 0.48 to 19.7 g/kg and 0.63 to 16.2 g/kg in the soil sampled in the Tuchola Forest. More TOC was found in the surface horizons of all the profiles, which had a medium-to-high content of humus (1.26–2.61%) (Table 1). According to Mocek and Owczarzak (2010), the humus content is low in Polish soils (in the Kuyavian-Pomeranian region, it is estimated to be 1.85%). The total nitrogen content ranged from 0.05 to 1.25 g/kg. The calculated distribution for this nutrient ( $DI > 6$ ) shows a marked tendency of very high levels of accumulation in the upper horizons of the soil profiles studied (close to the area affected by the factory), associated with an anthropogenic impact (Table 3). In the A horizon (0–20 cm) of the control site profile, the distribution ratio amounted to  $DI = 19$ . However, the main source of nitrogen in the control site soil could be from decomposing fallen tree leaves and vegetation, enriching it with nutrients. The ratio of carbon to nitrogen (C:N) was highest in the upper soil horizons (10.96–12.63 for profiles II–IV) and decreased in the deeper layers of the soil profile. According to Siuta (1995), a C:N ratio at a range 10:1 to 17:1 is slightly degraded soil. According to Brogowski and Chojnicki (2013), the vast majority of organic matter penetrat-

ing forest soils undergoes the process of mineralisation, whilst a smaller portion goes through the process of humification to result in humus. According to Kowalkowski and Kopron (2006), a narrow range of C:N ratio values indicates the excessive atmospheric deposition of nitrogen and sulphur, as well as particulate emissions rich in easily soluble Ca, Mg, K and S. The lowest value of C:N was found in soil profile I (9.37).

The amount of TP in the soil determines only the degree of its fertility and does not indicate its assimilability

level by vegetation. The amount of TP in the soil profiles affected by the factory was in the range 0.250–0.380 g/kg (Table 2), whilst at the control site, the amount was higher (0.308–0.412 g/kg). The value of the distribution ratio (*DI*) for TP indicates its pedogenetic accumulation.

The content of available phosphorus for plants in the surface horizons of the examined profiles was very low, ranging from 9.15 to 19.58 mg/kg (Table 2). This content was 19.44 mg/kg at a depth of 0–20 cm of the profile collected in Tuchola

**Table 1.** Physical and chemical properties of rusty forest soils

Profile No.	Depth [cm]	Horizon	C <sub>org</sub> g/kg	Humus %	N <sub>og</sub> g/kg	pH KCl	C:N	Fractions %			Symbol*
								0,05–2,0	0,002–0,05	<0,002	
I 0.8 km	0–15	A	7.31	1.26	0.78	4.46	9.37	87	12	1	ps
	15–72	Bv	4.09	0.71	0.41	4.99	10.0	90	9	1	ps
	72–100	C	0.72	0.12	0.12	4.09	6.00	97	2	1	pl
II 2 km	0–15	A	13.74	2.37	1.25	4.72	10.99	86	11	3	ps
	15–39	ABv	5.65	0.97	0.51	4.98	11.08	93	6	1	pl
	39–87	C1	0.38	0.07	0.05	4.97	7.60	84	12	4	ps
	87–100	C2	0.37	0.06	0.05	4.97	7.40	91	6	3	pl
III 2.5 km	0–4	A	15.1	2.61	1.20	4.39	12.63	87	6	7	ps
	4–18	ABv	9.05	1.56	0.75	4.93	12.07	97	2	1	pl
	18–60	Bv	3.09	0.53	0.31	5.17	9.97	95	3	2	pl
	60–150	C	0.40	0.07	0.05	5.41	8.00	97	2	1	pl
IV Control	0–20	A	12.5	2.16	1.14	5.11	10.96	88	3	9	ps
	20–50	ABv	3.80	0.66	0.37	5.13	10.27	95	0	5	pl
	50–100	BvC	0.53	0.09	0.07	4.25	7.57	94	1	5	pl
	100–130	C	0.49	0.08	0.06	4.31	8.17	98	1	1	pl

\*pl – loose sand; ps – slightly loamy sand

**Table 2.** The amount of total phosphorus (TP) and available phosphorus (P<sub>E-R</sub>), the activity of alkaline phosphatase (AIP) and acid phosphatase (AcP), the index of mobility (*IM*) and enzymatic soil pH index (*AIP/AcP*)

Profile No.	Depth [cm]	Horizon	TP g/kg	P <sub>E-R</sub> mg/kg	<i>IM</i> %	AIP	AcP	<i>AIP/AcP</i>
						mM pNP/kg/h		
I 0.8 km	0–15	A	0.281	9.156	3.25	1.445	12.87	0.11
	15–72	Bv	0.315	10.51	3.33	0.647	3.882	0.17
	72–100	C	0.259	6.233	2.40	0.310	0.670	0.46
II 2 km	0–15	A	0.314	11.89	3.78	1.581	6.578	0.24
	15–39	ABv	0.273	10.87	3.98	0.805	2.020	0.40
	39–87	C1	0.320	9.16	2.86	0.453	0.748	0.61
	87–100	C2	0.292	6.88	2.35	0.256	0.498	0.51
III 2.5 km	0–4	A	0.250	19.58	7.83	2.243	6.463	0.35
	4–18	ABv	0.258	14.73	5.71	0.913	1.783	0.51
	18–60	Bv	0.380	12.45	3.27	0.446	1.610	0.28
	60–150	C	0.268	7.239	2.70	0.223	0.546	0.41
IV Control	0–20	A	0.412	19.44	4.72	1.438	7.131	0.20
	20–50	ABv	0.308	15.41	5.00	0.719	2.308	0.31
	50–100	BvC	0.316	15.39	4.87	0.561	1.905	0.29
	100–130	C	0.321	8.53	2.65	0.179	0.776	0.23

Forest, as well as in study site III, 2.5 km away from the emissions. The critical value of available phosphorus for plants is about 30 mg/kg of soil. The lowest accumulation of  $P_{E-R}$  (6.233–10.51 mg/kg) was found in the profile located nearest to Anwil S.A. nitrogen works. It was associated with very acidic soil, as this element is particularly sensitive to changes in pH. At a low pH, part of the phosphorus forms slightly soluble compounds with the ions of Fe, Mn and Al, removing this element from biological use. At the same time, in the absence of fertilization, what little available phosphorus that remains is intensely absorbed by the vegetation, so that very small amounts of  $P_{E-R}$  remain in the soil. The index of mobility (*IM*) was used to assess the assimilability of phosphorus. The lowest *IM* value was found for soil profile I (3.25–2.40), which was associated with a low amount of  $P_{E-R}$  and confirmed by the analysis of correlation ( $r = 0.91$ ;  $p < 0.05$ ), whereas the highest *IM* value (2.7–7.82) was found for soil profile III.

The distribution index (*DI*) of available phosphorus in soil profile I was within a range 1.469–1.686; profile II from 1.332 to 1.728; profile III from 1.721 to 2.705, whilst the profile from Tuchola Forest was in the range 1.805–2.280 (Table 3), indicating moderate accumulation.

The content of available phosphorus decreased significantly as the depth increased of each soil profile, which should be associated with the low mobility of phosphorus in soil (Miletic et al. 2012). The research of Łabaz and Bogacz (2011) found a negative correlation of  $P_2O_5$  content with the depth of soil samples ( $r = -0.52$ ) and also indicated a low level of this element's leaching into the deeper layers of soil.

Enzyme assays allow an assessment to be made of the impact of both natural factors and human pressure on the functioning of ecosystems (Olszowska 2009; Bartkowiak, Lemanowicz 2014; Januszek et al. 2014). The activity of alkaline and acid phosphatases varied in the profiles examined depending on the distance from the emitter. In the soil profile collected approximately 0.8 m from the factory, the activity of alkaline phos-

phatase was inhibited (0.310–1.445 mM pNP/kg/h), whilst acid phosphatase activity was highest (0.670–12.87 mM pNP/kg/h) (Table 3). This was related to the high acidity of the soil. Higher acid phosphatase activity is due to the fact that phosphomonoesterases are enzymes that are most sensitive to changes in soil pH. The optimum pH for the activity of alkaline phosphatase is 9.0–11.0 and for acid phosphatase 4.0–6.5 (Wittman et al. 2004; Lemanowicz 2013). AIP activity in the control soil was in the range 0.179–1.438 mM pNP/kg/h, and AcP activity was in the range 0.776–7.131 mM pNP/kg/h. The high phosphorolytic potential of soils in pine plantations was shown by Bielińska et al. (2009). The studies of Mocek-Płóćiniak (2009) also confirmed the relationship between the activity of enzymes (dehydrogenase, phosphatase, urease and protease) and distance from a copper smelter in Legnica. High soil enzymatic inactivation at sites near the copper smelter was linked to the highest heavy metals pollution of the environment. Favourable changes in the studied soil parameters (TOC,  $P_{E-R}$ , AIP) with increasing distance from the nitrogen fertiliser factory are a sign of the soil's self-regulation capacity. At the same time, it should be emphasised that studied soil profiles were located in different directions from the emitter, thus the intensity of anthropogenic pressure could be related to the 'wind rose' (Bielińska, Ligęza 2010).

Phosphomonoesterase activity decreased as the depth of the soil profiles increased. This trend is related to the spatial distribution of humus, as well as soil microorganisms, and the declining amount of carbon substrates available for both microorganisms and enzymes (Januszek et al. 2006; Kizilkaya, Dengiz 2010), as confirmed by the results of TOC and humus amounts (Olszowska 2011; Brogowski, Chojnicki 2013).

Dick et al. (2000) used the measurement of phosphatase activity to determine optimal soil pH, as the ratio of alkaline to acidic phosphatase (the enzymatic indicator of pH level) proved to be a sensitive indicator of changes in soil pH. The *AIP/AcP* ratio in soil profiles located within the impact zone of the factory is in the range 0.11–0.61 (Table 2). According

**Table 3.** Distribution index value (*DI*)

Profile No.	Depth [cm]	Horizon	TOC	TN	TP	$P_{E-R}$
I 0.8 km	0–15	A	10.1	6.50	1.085	1.469
	15–72	Bv	5.68	3.41	1.216	1.686
II 2 km	0–15	A	37.1	25.0	1.075	1.728
	15–39	ABv	15.2	10.2	0.935	1.580
	39–87	C1	1.02	1.00	1.096	1.332
III 2.5 km	0–4	A	37.9	24.0	0.933	2.705
	4–18	ABv	22.6	15.0	0.963	2.036
	18–60	Bv	7.72	6.20	1.418	1.721
IV Control	0–20	A	25.5	19.0	1.283	2.280
	20–50	ABv	7.75	6.16	0.960	1.807
	50–100	BvC	1.08	1.16	0.984	1.805

to Dick et al. (2000), a ratio of *AIP/ACP* less than 0.5 indicates acidic soil. This was confirmed by the potentiometric measurements of soil pH in 1M KCl (Table 1). The lowest values of *AIP/ACP* ratio (0.11–0.35) were obtained from the surface layers of the studied profiles, which were most exposed to the emitter. The enzymatic indicator of the pH level can be used to determine the changes occurring in the soil (Dick et al., 2000; Lemanowicz 2013; Bartkowiak, Lemanowicz 2014).

On the basis of the statistical analysis, I obtained a nearly full correlation for the relationship between the TOC content and alkaline phosphatase activity ( $r = 0.94$ ;  $p < 0.05$ ) and a high correlation between TOC and acid phosphatase activity ( $r = 0.67$ ;  $p < 0.05$ ) (Table 4). The enzymatic activity directly depends on the amount of organic matter in the soil (An et al., 2008; Olszowska 2011).

**Table 4.** Person's correlation coefficients ( $n = 30$ )

Parameters	TOC	TN	C:N	P <sub>E-R</sub>	IM	AIP	AcP
TOC	-	0.99	0.84	0.68	0.69	0.94	0.67
TN		-	0.81	0.64	0.63	0.93	0.73
C:N			-	0.72	0.75	0.74	n.s.
P <sub>E-R</sub>				-	0.91	0.67	n.s.
IM					-	0.73	n.s.
AIP						-	0.78
AcP							-

Significance level  $p < 0.05$ ; n.s. - not significant.

High values of correlation coefficients between the studied enzymes and TOC and TN content indicate the importance of these enzymes in transforming the components of the organic matter found in the studied forest soils (Koper et al. 2008). Similar results were obtained earlier by Januszek et al. (2006). In contrast, the study by Bielińska et al. (2010) showed a close positive correlation between enzymatic activity (dehydrogenases, phosphatases and proteases) and amounts of  $N-NH_4^+$  and  $N-NO_3^-$ . This suggests that the ecosystem studied incorporated nitrogen compounds into its biological cycle reaching it from the atmosphere. A strong correlation was obtained between the amount of available phosphorus in the soil and the activity of alkaline phosphatase ( $r = 0.67$ ;  $p < 0.05$ ), suggesting that this enzyme was an appropriate parameter to use for characterising the amount of P<sub>E-R</sub> in the analysed soil, as opposed to the activity of acid phosphatase. A linear relationship is commonly observed between the activity of acid phosphatases and the amount of inorganic forms of phosphorus released into a soil solution (Nannipieri et al., 2011).

A significant positive correlation ( $r = 0.68$ ;  $p < 0.05$ ) was shown between the amount of available phosphorus and the amount of TOC. A higher amount of organic carbon compo-

unds usually results in increased biological activity of the soil, which accelerates the mineralisation of nutrients in the soil.

## 4. Conclusions

Based on the research conducted, the following conclusions were formulated:

1. Long-term emissions of nitrogen compounds increased soil acidity, decreased the soil's humus content and thereby reduced the amount of available phosphorus.
2. The values of the ratio of the distribution of phosphorus in the soil demonstrate its moderate accumulation, which is unrelated to the influence of anthropogenic pressure. In contrast, nitrogen accumulation was the result of anthropogenic activities.
3. The activity of alkaline phosphatase was found to have been greater as the distance from the nitrogen fertiliser factory increased and was accompanied by positive changes in the amounts of organic carbon compounds, humus and available phosphorus.
4. The tested parameters of forest soils varied depending on the distance from the emitter. The amount of total phosphorus, its available form and alkaline phosphatase activity increased with the distance from the nitrogen works. However, acid phosphatase activity decreased.

## Conflict of interest

The author declares no potential conflicts of interest.

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